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L3: Entry 19 of 54 File: USPT Jul 2, 2002

DOCUMENT-IDENTIFIER: US 6414127 B1
TITLE: Pyrimidine derivatives for labeled binding partners

Brief Summary Text (62):
Suitable amine salts include amines having sufficient basicity to form a stable salt, preferably amines of low toxicity including trialkyl amines (tripropylamine, triethylamine, trimethylamine), procaine, dibenzylamine, N-benzyl-betaphenethylamine, ephenamine, N,N'-dibenzylethylenediamine, N-ethylpiperidine, benzylamine and dicyclohexylamine.

Brief Summary Text (167):
The oligonucleotides of this invention contain naturally occurring nucleotides or derivatives thereof. In some oligonucleotide embodiments the companion nucleotide residues contain pyrimidine nucleotides substituted at the 5 position with a carbon atom which is distally Pi bonded to another atom as for instance 1-alkenyl, 1-alkynyl, heteroaromatic and 1-alkynyl-heteroaromatic groups such as 5-(1-propynyl)-cytosine and -uridine nucleotides (see PCT Publication No. WO 93/10820 and U.S. Pat. No. 5,594,121). Other analogs of native bases for use herein include alkylated purines or pyrimidines, acylated purines or pyrimidines, or other analogs of purine or pyrimidine bases and their aza and deaza analogs. These include, for example N.sup.4,N.sup.4 -ethanocytosine, 7-deazaxanthosine, 7-deazaguanosine, 8-oxo-N.sup.6 -methyladenine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, inosine, N.sup.6 -isopentenyl-adenine, 1-methyladenine, 2-methylguanine, 5-methylcytosine, N.sup.6 -methyladenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy aminomethyl-2-thiouracil, 5-methoxyuracil, pseudouracil, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-(1-propynyl)-4-thiouracil, 5-(1-propynyl)-2-thiouracil, 5-(1-propynyl)-2-thiocytosine, 2-thiothymidine, and 2,6-diaminopurine. In addition to these base analogs, one can conveniently incorporate into the invention oligonucleotides other base analogs, including pyrimidine analogs including 6-azacytosine, 6-azathymidine, 5-trifluoromethyluracil or other bases previously described, see, e.g., bases, monomers or oligonucleotides described in WO 92/02258, WO 97/32888 and U.S. Pat. No. 5,614,617.

Brief Summary Text (200):
Step one is conducted by heating the reaction mixture containing (100) in an organic solvent to at least about 50.degree. C., generally for about 3-4 hours. Step two is performed by reacting (102) in an organic solvent for about 6-48 hours, generally for about 10-20 hours at about 15.degree. C. to reflux temperature, generally at about 18-25.degree. C. The R.sup.2 moiety is linked under Mitsunobu conditions to (103) in step 3 by reacting about 1-1.5 equivalents of the alcohol, i.e., R.sup.2A --OH, using an activating agent as a leaving group, such as triphenylphosphine (Ph.sub.3 P) and diethyl diazocarboxylate (DEAD) to obtain (104). In step 4, (105) is prepared by forming the ring containing R.sup.47 by (1) incubating (104) in a polar organic solvent, typically an alkanol containing 1, 2, 3, 4, 5 or 6 carbon atoms, such as methanol or ethanol, containing a mild base such as NH.sub.3, TEA (triethylamine), DBU (1,8-diazabicydo[5.4.0]undec-7-ene) or (2) refluxing in ethanol in the presence of potassium fluoride. Generally (104) is incubated in saturated NH.sub.3 in methanol for about 2-3 days to afford (105).

Brief Summary Text (258):
One can also use the invention monomer compositions containing a 5' a-.sup.35 S-thiotriphosphate group or a 5' triphosphate group linked to 2',3'-dideoxyribose to perform dideoxy DNA sequencing methods. One may use invention monomers in kits that optionally contain buffers or enzymes suitable for DNA sequencing. The invention

monomers may be advantageously used in enzymatic DNA sequencing protocols because the invention monomers, which act as cytosine surrogates, have a high affinity for guanosine and may perform better than cytidine 5' triphosphate in sequencing reactions, particularly where the DNA to be sequenced contains a high proportion of guanosine residues, which can cause sequencing problems.

Brief Summary Text (265):
Invention oligonucleotides, including many structure (2), (2A), (2B) and (2C) oligonucleotides capable of forming high melting duplexes with complementary sequences, are useful in numerous applications, including antisense or codeblocking utilities in vivo or in vitro as well as diagnostics and probe uses. High melting duplexes are those having melting temperatures substantially above the melting temperatures of oligonucleotide or nucleic acid duplexes of the same sequence that contain the ordinary, naturally occurring bases, e.g., adenosine, cytidine, uridine, guanosine, thymidine and the like. "Substantially above" means that the derivative oligonucleotide, when hybridized with its complementary sequence, will not dissociate from the duplex until the temperature is raised from about 2 to 40.degree. C., ordinarily about 8 to 40.degree. C., above the dissociation temperature of the same oligonucleotide having the analogous normal A, C, U, G or T bases, but to no greater temperature than about 95.degree. C. This is known as the D Tm. Ordinarily, D Tm is measured by comparing control oligonucleotide binding to complementary RNA or DNA with the binding of test oligonucleotide to the same RNA or DNA, following, e.g., the method described in Jones et al., "JOC" 58:2983 (1993).

Detailed Description Text (26):
3'-5'-Diacetyl-N.sup.4 -(2",6"-dihydroxyphenyl)-2'-deoxy-5-bromo-cytidine (Compound #1154-093): A CCl.sub.4 --CH.sub.2 Cl.sub.2 solution (150 mL--150 mL) of 5-bromo-3'-5'-diacetyl-2'-deoxyuridine (15 g, 38.3 mmole) and Ph.sub.3 P (15 g, 57.5 mmole) was heated at reflux under N.sub.2 for 3 hrs. The reaction mixture was cooled to room temperature, followed by addition of 2-amino-resorcinol (5.2 g, 42 mmole) and DBU (8.7 g, 57.5 mmole). The resulting solution was stirred at room temperature overnight. The reaction mixture was concentrated to about 1/2 volume, then poured into citric acid aqueous solution (7.5 g in 300 mL H.sub.2 O) with vigorously stirring. The precipitate was filtered off, washed with H.sub.2 O, CH.sub.2 Cl.sub.2 then CH.sub.3 CN, dried in a vacuum oven overnight, weighed 12.9 g, 67.8% yield of title compound. .sup.1 H NMR (DMSO-d.sub.6): .delta. 9.63 (s, 2H), 8.21 (s, 1H), 8.0 (s, 1H), 6.89 (t, 1H, J=8.1 Hz), 6.33 (d, 2H, J=8.1 Hz), 6.10 (t, 1H, J=7.4 Hz), 5.10-5.17 (m, 1H), 4.12-4.30 (m, 3H), 2.30-2.40 (m, 2H), 2.06 &2.03 (2s, 6H).

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L3: Entry 48 of 54 File: USPT Mar 11, 1997

DOCUMENT-IDENTIFIER: US 5610292 A
TITLE: Process for producing 2,2'-o-cyclonucleosides nucleosides, and analogs thereof

Brief Summary Text (5):
Ogilvie (Carbohyd. Res., 24, 210 (1972)) teaches the production of cytanbine from cytidine. Specifically, the process comprises reacting cytidine with diphenyl carbonate and sodium hydrogen carbonate at 150.degree. C in DMF. The product cytarabine was purified using thin layer chromatography and obtained in a yield of 40%.

Brief Summary Text (6):
Beranek et al (Nucleic Acid Chemistry, Vol. 1, 249, Edited by Townsend and Tipson, Wiley, N.Y.) teach the production of cytarabine from cytidine. Specifically, cytidine is reacted with incremental amounts of diphenyl carbonate in the presence of DMF and water at 120.degree. C. The overall yield of pure cytarabine was limited to 31.9%.

Brief Summary Text (7):
Roberts et al (J. Org. Chem. 32, 816 (1967)) teach the production of cytarabine from cytidine (or from 2'(3')-cytidylic acid). Specifically, cytidine is reacted with phosphoric acid at 80.degree. C. for a period of 30 hours to produce a 2,2'-O-cyclocytidine analogue intermediate. This intermediate is then hydrolyzed at a pH of 9 utilizing lithium hydroxide to produce the 3',5'-diphosphate of cytarabine. The diphosphate is then treated with magnesium chloride, ammonium chloride and concentrated ammonium hydroxide, and thereafter purified by column chromatography to yield pure cytarabine. The overall yield of pure cytarabine is limited to 53% based on the unrecovered portion of the starting cytidine.

Brief Summary Text (10):
Further, the production of cyclonucleosides is known. For example, Walwick et al (Proc. Chem. Soc., 84 (1959)) teach the production of 2,2'-O-cyclocytidine hydrochloride from cytidine. The process involved heating cytidine with polyphosphoric acid followed by dephosphorylation of one of the reaction products, 2,2'-O-cyclocytidine-3',5'-diphosphate.

Brief Summary Text (11):
Doerr et al (J. Org. Chem., 32, 1462 (1967)) teach the production of 2,2'-O-cyclocytidine chloride from uridine using a process comprising six steps. It is interesting to note that in the final step, 2,2'-O-cyclotidine hydrochloride was obtained only in a 57% yield. Taking into account the fact that each step is not quantitative, the overall yield of 2,2'-O-cyclocytidine hydrochloride from uridine can be expected to be on the order of from 10% to 20%.

Brief Summary Text (12):
Kikugawa et al (Tet. Lett., 869 (1970)) teach the production of the hydrochloride or the formate salt of 2,2'-O-cyclocytidine. Specifically, the process comprises reacting cytidine with thionyl chloride and N,N'-dimethylfonnamide. It is interesting to note that the crude 2,2'-O-cyclocytidine salt was obtained in a yield of only 30.4%. Kikugawa et al (J. Org. Chem., 37, 284 (1972)) also provide an improved process for preparing 2,2'-O-cyclocytidine. The improvement appears to relate to an improved yield (55%) of the product using ion exchange and chromatography techniques.

Brief Summary Text (14):
Yamaguchi et al (J. Med. Chem., 19, 654 (1979)) teach the production of

2,2'-O-cyclocytidine hydrochloride via reaction of cytidine with an organic acid chloride.

Brief Summary Text (36):
Preferably, the process for producing a compound of Formula II can be used to produce 2,2'-O-cycloribonucleosides such as 2,2'-O-cyclocytidine, 2,2'-O-cyclouridine, 2,2'-O-cyclothymidine, or pharmaceutically acceptable salts thereof. Generally, 2,2'-O-cycloribonucleosides may be prepared by reacting the appropriate nucleoside with the appropriate dialkyl tin oxide. More preferably, this process is used to produce 2,2'-O-cyclocytidine by reacting a cytidine-compound-tin oxide conjugate of Formula III in which W is --N.dbd.C(NH.sub.2)-- and Z is hydrogen.

Brief Summary Text (37):
In one preferred embodiment of the invention, in which the compound of Formula III is a cytidine conjugate, R.sup.5 is butyl and R.sup.1 is hydrogen. With these definitions for R.sup.5 and R.sup.1, the compound of Formula III is 2',3'-O-dibutylstannylene cytidine.

Brief Summary Text (39):
Provided that it does not contain a hydrogen bonded to nitrogen, the amine suitable for use in the process for producing a compound of Formula II is not particularly restricted and may be selected from the group comprising trimethylamine, triethylamine, pyridine, tripropylamine and tributylamine. The most preferred amine is triethylamine.

Brief Summary Text (44):
wherein R.sup.2, R.sup.3 and R.sup.4 can be the same or different and are selected from the group comprising hydrogen, a C.sub.1 -C.sub.6 alkyl groups and a C.sub.6 -C.sub.9 aryl group, with the proviso that each of R.sup.2, R.sup.3 and R.sup.4 is not hydrogen. Thus, it will be appreciated that the use of ammonia (i.e. R.sup.2 =R.sup.3 =R.sup.4 =H) is outside the scope of the present invention. Non-limiting examples of suitable heterocyclic amines include pyridine and piperidine. Non-limiting examples of other amines suitable for use include t-butylamine, trimethylamine, triethylamine, tripropylamine, tributylamine, methylamine, ethylamine, diethylamine and aniline. The most preferred amine suitable for use in the present process is t-butylamine.

Detailed Description Text (3):
A 500 mL flask was charged with 50 mL methanol, 1.95 g cytidine and 2 g dibutyl tin oxide. The resulting suspension was refluxed for five hours and then stirred at room temperature for twelve hours. To the mixture was then added triethylamine (7.8 mL) followed by slow addition of p-toluenesulfonyl chloride (10.68 g). The resulting mixture was stirred for twelve hours at room temperature. Thereafter, the solvents were evaporated under vacuum and chloroform (100 mL) was added to the resulting white gum. The chloroform/white gum suspension was refluxed for fifteen minutes and then cooled to room temperature. The resulting white precipitate was filtered and washed with chloroform, and dried to yield 1 g of crude 2,2'-O-cyclocytidine hydrochloride. The crude cyclocytidine hydrochloride was suspended in 5 mL water and the mixture was heated to 60.degree. C. This solution was filtered and the solvent reduced under vacuum to obtain a turbid oil. Ethanol (18 mL) was added and the mixture was stirred at 5.degree. C. for twelve hours. The resulting precipitate was filtered and dried to provide 0.6 g of pure 2,2'-O-cyclocytidine hydrochloride (29% yield). The product was characterized by comparison of its melting point, and NMR and IR spectra with those previously reported for 2,2'-O-cyclocytidine.

CLAIMS:

5. The process defined in claim 4, wherein said amine is triethylamine.

8. The process defined in claim 1, wherein said amine is selected from the group consisting of trimethylamine, triethylamine, pyridine, tripropylamine and tributylamine.

14. The process defined in claim 11, wherein the amine used in step (ii) is selected

from the group consisting of trimethylamine, triethylamine, pyridine, tripropylamine and tributylamine and the amine used in step (iv) is selected from the group consisting of t-butylamine, tzimethylamine, triethylamine, pyridine, tripropylamine, tributylamine, methylamine, ethylamine, diethylamine, aniline and piperidine.

15. The process defined in claim 14, wherein the amine used in step (ii) is triethylamine and the amine used in step (iv) is t-butylamine.

23. The process defined in claim 18, wherein said amine is selected from the group consisting of trimethylamine, triethylamine, pyridine, tripropylamine and tributylamine.

24. The process defined in claim 22, wherein said amine is triethylamine.